

Differences in the Vector Efficiency of *Thrips tabaci* in Europe and North America

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Although *Thrips tabaci* is a well-known vector of *Tomato spotted wilt virus* (TSWV) it does not belong to the spreaders of this dangerous pathogen in North America. The possible explanation of the differences in its vector efficiency in Europe and in North America is rooted in the fact that out of the two subspecies of *T. tabaci*, i.e. *T. tabaci tabaci* and *T. tabaci communis* only the specimens of the latter were introduced from Europe into North America. To support our hypothesis we have used a molecular marker that detects intraspecific ribosomal DNA sequence variations between the two subspecies of *T. tabaci*.

Keywords: *Thrips tabaci tabaci*, *Thrips tabaci communis*, TSWV, different vector efficiency.

Thrips tabaci was the first published vector of *Tomato spotted wilt virus* (TSWV) (Pittmann, 1927) and has been known to be effective in spreading this virus disease, causing severe epidemics on tobacco, pepper, tomato and ornamental plants all over Europe (Razvyazkina, 1953; Sęczkowska, 1969; Gáborjányi et al., 1993; Lemmetty and Lindqvist, 1993; Asjes and Blom-Barnhoorn, 1997; Chatzivassiliou et al., 2001), in Australia (Norris, 1951; Latham and Jones, 1997) and Hawaii (Sakimura, 1932). At the same time its populations are not mentioned among the vectors of TSWV in North America (Paliwal, 1974, 1976; McPherson et al., 1992, 1999; Eckel et al., 1996). According to the opinion of Ullman (1996) and Chatzivassiliou (2002) the worldwide distribution of *T. tabaci* may have resulted in a large divergence in its competence to transmit TSWV.

Although the vector activity of *T. tabaci* was first published by Pittmann (1927), it is noteworthy to mention that during investigations on the causal agent of a severe damage in tobacco Lindeman observed the occurrence of *T. tabaci* in high population density in Bessarabia already in 1889. At the same time, Lindeman (1889) has described a symptom that appeared on tobacco leaves infested by *T. tabaci*, which later was identified as the damage caused by TSWV (Zawirska, 1976). It is likely that Lindeman's was the first observation of the joint presence of TSWV and *T. tabaci* on tobacco. The transmission of TSWV by *T. tabaci* was first established in Europe namely in the Soviet Union by Razvyazkina in 1953. Subsequently the occurrence of TSWV vectored by *T. tabaci* was

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recorded from Poland (as *Lycopersicum virus 3*) (Sęczkowska, 1969), Hungary (Gáborjányi et al., 1993), Finland (Lemmetty and Lindqvist, 1993), Czech Republic (Mertelik et al., 1996), The Netherlands (Asjes and Blom-Barnhoorn, 1997) and Greece (Chatzivassiliou et al., 2001). According to these data, TSWV vectored by *T. tabaci* has been a well-known virus disease of tobacco as well as of pepper, tomato, and ornamental plants in Europe.

TSWV causes severe yield losses in North America, too. To prevent TSWV epidemics the vector efficiency and activity of some Thysanoptera species were thoroughly investigated in North Carolina (Eckel et al., 1996) and Georgia (McPherson et al., 1992, 1999) but *T. tabaci* was not mentioned among the species investigated. According to the opinion of Paliwal (1974, 1976) the specimens of *T. tabaci*, are not able to transmit TSWV in Canada, due to the lack of compatible isolates. However, it is important to consider that *T. tabaci* frequently occurs in high population density in Europe on tobacco having been introduced from South America (Zeven and Zhukovsky, 1975). At the same time, *T. tabaci* introduced from Europe is not mentioned among the pests of tobacco in North Carolina (Reddy and Wightman, 1988; Eckel et al., 1996) and in Georgia (McPherson et al., 1995, 1999). According to Reddy and Wightman (1988) *Frankliniella fusca* feeds on tobacco in North America but does not transmit the virus. On the other hand, *T. tabaci* feeds on tobacco in Russia and the Balkans but does not in North America. In North America *Frankliniella fusca* (Hinds) is known under the common name of tobacco thrips (McPherson et al., 1995). However, *T. tabaci* frequently occurs in high population density on onion (Shirck, 1951; Boyce and Miller, 1954; Stannard, 1968), leek and garlic (Bailey, 1938) and cabbage (North and Shelton, 1986a, 1986b; Shelton and North, 1986) causing severe damage in the USA.

The gene centre of *Allium cepa* (onion) and *A. sativum* (garlic) is located in Central Asia, while that of *A. porrum* (leek) in the Mediterranean (Zeven and Zhukovsky, 1975), therefore, it is possible that this area is the true homeland of *T. tabaci* (Mound, 1983). Since the occurrence and propagation on tobacco were observed by Lindeman (1889) in Bessarabia (Europe), it is remarkable, that the gene centre of *Nicotiana tabacum* (tobacco) and *N. rustica* (Makhorka) is located in South America (Zeven and Zhukovsky, 1975), from where it was introduced into Europe in 1556 (Natter-Nád, 1939). Consequently, the populations of *T. tabaci* could feed and propagate on tobacco only for some 400 years.

While investigating the causes of the different vector efficiencies of *T. tabaci* the existence of two types: *T. tabaci communis* and *T. tabaci tabaci* was established by Zawirska (1976, 1978).

– Only females are members of the populations of the type *T. tabaci communis*. It propagates by thelytokous parthenogenesis and the offspring of unfertilised females are females. Its populations have a wide range of breeding plants, are serious pests of many plants, mainly onion, garlic, leek and cotton, and are not capable of transmitting TSWV.

– Both females and males are present in the populations of the type *T. tabaci tabaci*. It propagates by arrhenotokous parthenogenesis and its populations are associated particularly with tobacco, and are capable of transmitting TSWV.

The occurrence of the arrhenotokous populations on *Allium* spp. (Shull, 1914; Harris et al., 1936; Mound, 1983; Kendall and Capinera, 1990; Torres-Vila et al., 1994; Vierbergen and Ester, 2000; Jenser et al., 2006) does not confirm the statement of Zawirska

(1978) regarding the different range of breeding plants of arrhenotokous and thelytokous populations. However, a distinct genetic differentiation of leek-associated and tobacco-associated *T. tabaci* populations has been demonstrated. It is presumable that the initial divergence into leek- and tobacco-associated clades occurred around 28 million years ago (Brunner et al., 2004). In fact, this report verified Zawirska's (1976) statement regarding the different range of breeding plants of *T. tabaci communis* and *T. tabaci tabaci* types. Furthermore, experiments conducted by Wijkamp et al. (1995) and Chatzivassiliou (2002) confirmed Zawirska's findings that populations of the *T. tabaci tabaci* type (i.e. tobacco-associated populations) are the efficient vectors of TSWV.

Our hypothesis is that the difference in vector efficiency of *T. tabaci* in Europe and North America is due to the different geographical distribution of the two subspecies (*T. tabaci tabaci* and *T. tabaci communis*). It is likely that only specimens of the latter were introduced from Europe into North America. We present evidence supporting our hypothesis by employing a molecular marker that detects intraspecific ribosomal DNA sequence variations between the two *T. tabaci* subspecies.

Materials and Methods

Data of 1400 slides of *T. tabaci* specimens deposited in The National Collection of Thysanoptera in the USDA Systematic Entomology Laboratory in Beltsville MD have been investigated. These specimens were collected from plants imported into the USA, as well as from plants cultivated in North America during the 20th century.

Thrips tabaci specimens were collected for PCR examinations from:

- tobacco (*Nicotiana tabacum*) in Hungary
- onion (*Allium cepa*) in Hungary and in USA
- cabbage (*Brassica oleracea*) in Hungary and in USA

DNA extraction, amplification, cloning and sequencing

Total genomic DNA was extracted from single individuals using REDExtract-N-AmplTM Tissue PCR Kit (Sigma) according to the manufacturer's instructions. *Thrips tabaci* females were collected from tobacco, onion and cabbage originated from the USA and Hungary, 3–5 individuals from each group were tested. After preliminary studies the primer pair CASSp8Fc and CAS28sB1d (Kim and Lee, 2008) was selected for PCR. The primers amplified an ITS 2 sequence of nuclear DNA. PCR was performed using *Taq* DNA polymerase (Fermentas) in a thermo-cycler (Eppendorf Mastercycler gradient) according to the following procedure: initial denaturation at 96 °C for 4 min, followed by 40 cycles of 95 °C for 30 sec, annealing at 50 °C for 30 sec, extension at 72 °C for 60 sec; final extension at 72 °C for 10 min. The PCR products were purified using the Gel/PCR DNA Fragments Extraction Kit (Geneaid). Purified PCR products from 3 individuals of *Thrips tabaci* females per each group were cloned into a CloneJet (Fermentas) vector and inserted into *Escherichia coli* DH5 α competent cells. All cloning steps were based upon standard molecular biology protocols (Sambrook et al., 1989). The recombinant plasmids isolated from selected colonies were sequenced using pJET1.2 forward and reverse primers, the PCR

products were sequenced by CAS5p8sFc and CAS28sB1d primers by an automated DNA sequencer (Applied Biosystem Gene Analyzer 3100). Sequence comparisons were performed using the Wisconsin Package version 10.0 Genetic Computer Group (GCG) sequence analysis software (Devereux et al., 1984). DNA sequences in the ITS2 region of the thrips specimens were deposited to the GenBank (*Table 1*).

Table 1

List of specimens, geographical origins, host plants and GenBank Accession numbers

Specimen code number	Location	Host plant	GenBank Access. No.
A51.5	Hungary	onion	JF968500
A51.7	Hungary	onion	JF968505
A61.4	Hungary	cabbage	JF968494
A56.10	USA	onion	JF968504
A56.13	USA	onion	JF968503
A56.7	USA	cabbage	JF968502
A61.9	USA	cabbage	JF968492
A61.15	USA	cabbage	JF968493
A56.6	Hungary	tobacco	JF968501
A72.11	Hungary	tobacco	JF968498

Results

Onions have been transported from Europe to North America for a long time (Brewster, 1994). *Thrips tabaci* specimens collected from onions imported from France (7 slides), from Italy (10 slides), from Spain (4 slides) are deposited in the National Collection of Thysanoptera in the USDA Systematic Laboratory in Beltsville MD. It is clear that transmission of the specimens of *T. tabaci communis* into North America has probably been continuous for many hundred years.

The fact that in North America *T. tabaci* is a common pest on onion and cabbage, (Shelton et al., 1982; North and Shelton, 1986a, 1986b; Shelton and North, 1986) but does not occur on tobacco (Reddy and Wightman, 1988; Eckel et al., 1996; McPherson et al., 1995, 1999) and in the National Thysanoptera Collection there is no *T. tabaci* collected from tobacco, is a further evidence that specimens of *T. tabaci communis* were introduced and spread in North America.

Nucleotide sequence homology in the internal transcribed spacer 2 (ITS 2) region of the nuclear ribosomal DNA extracted from thrips specimens were compared to each other. Nucleotide sequence identity varied between 95.8 and 99.8% (*Table 2*). Identity values among *Thrips tabaci* specimens originated from onion or cabbage were between 99.0–99.8%, independently of the collection locality (i.e. USA or Hungary). However, nucleotide sequence identity for *Thrips tabaci* specimens collected from tobacco in Hungary compared to all other specimens proved to be much lower, in the range of 95.8–97.3%.

Table 2

DNA sequence homology (percent of identity) in the ITS2 region of *Thrips tabaci* specimens originating from different host plants

		Hungary		USA				Hungary	
		onion	cabbage	onion		cabbage		tobacco	
		A51.7	A61.4	A56.10	A56.13	A56.7	A61.9	A61.15	A56.6 A72.11
Onion	A51.5	99.4	99.8	99.8	99.4	99.8	99.2	99.8	96.5 97.3
	A51.7		99.2	99.2	99.2	99.2	99.0	99.2	95.8 96.7
Cabbage	A61.4			99.6	99.2	99.6	99.0	99.6	96.1 96.7
Onion	A56.10				99.2	99.6	99.0	99.6	96.3 97.1
	A56.13					99.2	99.0	99.2	95.8 96.7
	A56.7						99.2	99.6	96.5 97.1
Cabbage	A61.9							99.2	96.1 96.7
	A61.15								96.5 97.3
Tobacco	A56.6								99.0

Conclusions

It is very likely that of the two subspecies of *T. tabaci* only the specimens of *T. tabaci communis* (Zawirska, 1976) named also as onion associated populations (Brunner et al., 2004) were introduced into North America.

Our results gained by analysing nucleotide sequences in the ITS2 region seem to support this hypothesis; *Thrips tabaci* specimens collected in North America had high identity values to those originated from onion or cabbage from Europe, in fact, they were almost identical. On the other hand, all specimens collected from onion or cabbage differed significantly from the Hungarian/European *T. tabaci tabaci*.

According to the available data, only the specimens of *T. tabaci communis* populations had spread in North America, which are not capable to transmit TSWV. This is the reason why *T. tabaci* is not a vector of TSWV in North America at present. However, nowadays, when intercontinental transport takes only a few hours, it will have become possible to introduce the specimens of *T. tabaci tabaci* overseas. As a consequence, *T. tabaci* could eventually become a vector of TSWV also in North America.

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